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14. ABSTRACT

Prostate cancer is a major health concern in the United State and understanding the molecular biology underlying the development of prostate cancer can help improve the disease prevention and therapeutic strategies. Steroid receptor coactivator 3 (SRC-3) is a nuclear receptor coactivator that is important for growth of endocrine tissues. SRC-3 enhances proliferation of prostate cancer cell lines in a cell-autonomous manner and its expression is highly correlated with aggressiveness of human prostate cancer tumor samples. Here I use animal models to study the function of SRC-3 in prostate cancer and ascertain the role of SRC-3 in a cell-type specific manner by employing the lox-Cre knockout system. In the first part of my research funding period, I aimed to determine whether SRC-3 promotes prostate tumor progression in cancer derived from luminal epithelial cells (LECs) by simultaneously deleting the Pten and SRC-3 genes in the LECs of the mouse prostate. I also assessed the function of SRC-3 in castration resistant prostate cancer (CRPC) by performing androgen deprivation experiments on this mouse model. I found that deletion of SRC-3 caused impairment of cellular proliferation translating into a progressive decrease in tumor size. While double knockout tumors did not histologically appear less disorganized or invasive, they exhibited a relative increase in basal-like and decrease in luminal-like cells. When I performed androgen-deprivation assay, I found that castration induces significant changes in the phenotype of tumors evidenced by cellular de-differentiation and stromal reactivity. SRC-3 deletion results in reversal of the castration-induced changes accompanied by a decreased S6 kinase in the tumor indicating a decrease in cellular translational output. This result provides a potential mechanistic link between SRC-3 absence and decreased tumor growth. In summary, SRC-3 controls the size of the prostate tumor and is a critical mediator in the development of CRPC.

15. SUBJECT TERMS

Prostate cancer, Castration-resistant prostate cancer, steroid-receptor coactivator-3

Table of Contents

INTRODUCTION:

Prostate cancer affects one in six American men and is the second leading cause of cancer death in this population (ACS, 2010). Advanced disease, in which cancer invades adjacent structures and gains metastatic capacity, is the primary cause of prostate cancer-related mortality. Steroid receptor coactivator 3 (SRC-3) is a nuclear receptor coactivator that is important for growth of endocrine tissues (1). SRC-3 enhances proliferation of prostate cancer cell lines in a cell-autonomous manner and its expression is highly correlated with aggressiveness of human prostate cancer tumor samples (2,3). In addition, prostate cancer cell line studies have revealed that SRC-3 serves as a coactivator for AP1 transcription factor, promoting cell proliferation through the IGF/Akt pathway (4). Here we use animal models to study the function of SRC-3 in prostate cancer and ascertain the role of SRC-3 in a cell-type specific manner by employing the lox-Cre knockout system. In the first part of my research funding period, I aimed to determine whether SRC-3 promotes prostate tumor progression in cancer derived from luminal epithelial cells (LECs) by simultaneously deleting the Pten and SRC-3 genes in the LECs of the mouse prostate. In the second part, I will ascertain whether SRC-3 promotes prostate cancer progression in tumors arising from basal cell progenitors by simultaneously deleting SRC-3 and Pten genes in the basal cells of mouse prostate using a similar strategy. I also assess the function of SRC-3 in castration resistant prostate cancer by performing androgen deprivation experiments on these two mouse models. The results from this research study will shed light into the potential of SRC-3 inhibitors as ovel therapeutic agents for treating advanced prostate cancer. Specifically, we will learn from this study which epithelial cell type is prone to tumorigenesis and if SRC-3 ablation can inhibit the tumorigenesis from this cell type.

BODY:

Specific Aim 1: Assess the oncogenic role of SRC-3 in luminal epithelial cells by simultaneously deleting floxed PTEN and SRC-3 genes via Probasin (ARR2PBi)-Cre.

Task 1: Generate experimental pten$^{f/f}$/SRC3$^{f/f}$/ARR2PBiCre (pten3cko) and control pten$^{f/f}$/ARR2PBiCre (ptencko) (months 1-9)

I successfully generated experimental and control mice during the first 9 months of the funding period. The mice were sacrificed at the following ages: 6, 9, 12, 18 and 24 weeks. The number of mice at each time point was 10. Here, we employed the ARR2PBi Cre to simultaneously delete floxed Pten and SRC3 genes in prostatic epithelial cells. Pten/SRC-3 double knockout mice are termed pten3cko, while single Pten knockouts are termed ptencko. As shown in figure **(1A-B)**, SRC-3 is deleted in virtually all prostatic luminal cells of pten3cko mice but retained in some basal cells.

Task 2: Assess prostate cancer progression and metastasis in these mice

a) I assessed the impact of SRC-3 deletion on tumor growth by comparing weights of pten3cko and ptencko prostates harvested at 6, 9, 12, 18 and 24 weeks of age **(1C)**. Indeed, prostate weight was lower by more than 50% in pten3cko vs. ptencko mice at 18 and 24 weeks **(1C)**. Tumor growth in the anterior lobe principally accounted for this dramatic difference in tumor size **(1D-E)**. On the contrary, tumor histology was not markedly different between groups, with the exception of an increase in large cystic spaces seen in ptencko mice **(1F-G)**.

b) I observed nearly twice as many Ki67(+) cells in ptencko mice vs. pten3cko mice **(2A-B)**, translating into proliferation indices of 9% and 5%, respectively **(2E)**. Intriguingly, co-staining with SRC-3 showed virtually all Ki67(+) cells in ptencko mice co-expressed SRC-3 **(2C-D)**. I performed TUNEL assay to quantify apoptosis but found no significant difference between the groups (data not shown). Next, I performed immunohistochemistry for specific biomarkers. Pten3cko tumors had a dramatic increase in the number of p63(+) basal cells vs. ptencko mice **(2F-H)**. This correlated with an increase in staining for cytokeratin-5 (K5), a marker of the basal layer **(2I-J)**. Conversely, staining for cytokeratin-8 (K8), a marker of the luminal layer, was substantially lower in pten3cko mice **(2I-J)**. No change was observed in androgen receptor (AR) distribution **(2K-L)**. In sum, SRC-3 knockout yields smaller tumors with lower proliferation index and a specific decrement in luminal cells. This is coupled with relative expansion of the basal compartment.

c) Metastases to lymph nodes, lung and liver in ptencko and pten3cko mice were assessed. However, only metastases to lymph nodes were observed. No metastases to lung and liver were observed in these mouse models.

d) I isolated RNA and protein from tumor samples. I carried out the molecular analysis at the same time as the samples from Task 3 were collected.

Task 3: Perform androgen deprivation assay to determine SRC3 function in castration-resistant prostate cancer

a) To determine if SRC-3 functions to promote CRPC, I wanted to first set up a model to evaluate CPRC in the setting of Pten deletion. I performed surgical castration on 9-wk old ptencko mice and harvested tissues at 12-wks. We found castration made tumors more histologically aggressive, with increased stromal infiltration and cellular proliferation (Figure 3). I then performed surgical castration on ptencko and pten3cko mice. 10 mice from each genotype were castrated at 9 weeks of age and sacrificed at 12 weeks of age.

b) All the tumor assessments were carried out similarly to Task 2. As indicated earlier, SRC-3 deletion in Pten-null mice caused reduction in prostate tumor size, proliferation index and luminal epithelial marker staining. However, histological comparison of aggressiveness and invasiveness did not show significant differences between ptencko and pten3cko mice. In order to examine the role of SRC-3 in CRPC, I castrated both groups of mice at 9 weeks of age, a time point prior to the development of adenocarcinoma. At 9 weeks, tumors of ptencko and pten3cko mice were similar in weight and histologically identical, consisting only of low PIN **(4A-B).** SRC-3 immunohistochemistry confirmed efficiency of knockout system, demonstrating absence of SRC-3 in virtually all luminal epithelial cells **(4D)**. In keeping with the similarity in tumor size, Ki67 proliferation index was comparable in ptencko and ptne3cko mice **(4E-G)**. In sum deletion of SRC-3 has no impact on tumor histology or cellular proliferation in non-castrated mice at 9wks. Therefore, this represents a suitable time point to perform surgical castration on ptencko and pten3cko mice in order to assess CRPC **(5A)**. We analyzed tumor samples at 12 weeks of age (3 weeks post-castration) and found that tumors from pten3cko mice were much smaller than those of ptencko mice **(5B-C)**. The relative weight of pten3cko prostates was only 30% that of ptencko prostates **(5D)**. SRC-3 immunohistochemistry indicated SRC-3 was highly expressed in castrated ptencko tumors but absent in virtually all LECs of castrated pten3cko tumors **(5E-F)**. **(5E-F)** also demonstrate a significant difference in the histology between the two groups. In the ptencko tumor, histology was disorganized with substantial stromal infiltration. On the contrary, sections from pten3cko samples resembled the tumor histology prior to castration. Ki67 immunohistochemistry also demonstrated more proliferating cells in castrated ptencko vs. castrated pten3cko tumors **(5G-H)**. Statistical analysis showed Ki67 proliferation index of pten3cko tumor was reduced to only a quarter of that seen in ptencko tumors **(5I)**. In conclusion, deletion of SRC-3 further reduced tumor size and prevented transition from a benign to an aggressive tumor during development of CRPC.

c) To further characterize these tumors, we performed immunohistochemistry for differentiation markers and stromal reactivity indicators. We demonstrated that castration caused an increase in the number of p63 (+) cells in the ptencko tumor. Double staining of p63 and SRC-3 in castrated ptencko tumos revealed clusters of p63 (+) cells, the majority of which co-express SRC-3 **(6A-B)**. On the contrary, in castrated pten3cko tumors, the number of p63 (+) cells was much lower, and no colocalization with remnant SRC-3(+) cells was visible. In addition, AR expression was higher in pten3cko vs. ptencko tumors, indicating cancer cells in pten3cko animals were more differentiated **(6C-D)**. We also compared stromal reactivity between tumors of the two groups. Vimentin/SMA double staining is a measure of reactive stroma. Increased expression of both Vimentin (green) and SMA (red) indicated higher stromal reactivity in ptencko tumors **(6E).** Furthermore, while SMA distribution was disorganized in ptencko tumors, it showed an organized monolayer surrounding the ductal glands in pten3cko tumors **(6F)**. Trichrome

staining also revealed significant collagen deposits in ptencko tumor **(6G)** while none were present in pten3cko tumors **(6H)**. Other cancer markers such as E-Cadherin and CD31 further substantiate a less aggressiveness phenotype pten3cko tumor vs. ptencko tumors. E-Cadherin, an epithelial marker, was higher in pten3cko than ptencko tumor **(6I-J)** while CD31, which indicates angiogenesis, demonstrated more blood vessel formation in ptencko tumor than pten3cko **(6K-L)**. In conclusion, ablation of SRC-3 prevents development of CRPC, indicating the cellular proliferation, de-differentiation and stromal reactivity that characterize this tumor type depend on SRC-3 action.

e) I isolated RNA and protein from the castrated prostate tumors and carried molecular analyses. I found that observed no differential expression in any of the tested AR targets and, in fact, found transcript levels significantly lower than wild-type prostate in both tumor groups. This confirms prior reports of suppressed AR signaling in the Pten knockout mouse and suggests SRC-3 promotes tumor growth by another mechanism. The mTOR pathway is a critical mediator of cell growth and proliferation that is upregulated in a number of cancers. SRC-3 has been shown in cell lines to promote mTOR signaling. In tumors of ptencko and pten3cko mice, we analyzed phosphorylated S6 as an assay of mTOR pathway activity. Immunohistochemistry demonstrated that pS6 was widely distributed in the prostatic epithelium of ptencko mice but virtually absent in that of pten3cko mice **(7A-B)**. Western blots performed on whole-tumor lysates confirmed this finding **(7C)**. S6 is phosphorylated by S6 kinase (S6K), a protein with both cytosolic (p70) and nuclear (p85) isoforms. We found that, while the level of cytosolic S6Kp70 is unchanged between ptencko and pten3cko mice, the level of nuclear S6Kp85 is significantly lower in pten3cko mice **(7C)**. In parallel, the level of S6K mRNA is reduced in pten3cko mice **(7D)**. In sum, SRC-3 regulates S6 phosphorylation by influencing both the transcription and protein isoform distribution of S6K.

KEY RESEARCH ACCOMPLISHMENTS

- We successfully generated mice in which floxed PTEN and SRC-3 genes were concomitantly deleted in prostate luminal epithelial cells via a Cre recombinase driven by the Probasin promoter. This mouse model was necessary to study the function of SRC-3 in promoting prostate cancer.
- We found that SRC-3 knockout yields significant changes in prostate tumor weight at late stages. Deletion of SRC-3 decreases tumor proliferation and changes cellular composition of the tumor.
- We found that castration increases tumor aggressiveness and cellular proliferation in prostate cancer caused by Probasin-mediated Pten deletion and that tumors from castrated mice have a de-differentiated phenotype with increased stromal reactivity
- We found that deletion of SRC-3 significantly reduces tumor size and cellular proliferation in castration-resistant prostate cancer and deletion of SRC-3 reverses castration-induced changes in tumor cell type and stromal reactivity
- We found S6K translational pathway is enhanced by SRC-3 in castration-resistant prostate cancer.

REPORTABLE OUTCOMES:

Publications

Tien JC, Liu Z, Gao L, Wang F, Xu J. "SRC-3 is a critical mediator in the development of castration-resistant prostate cancer." (Manuscript in preparation)

Poster Presentation

Tien JC, Liu Z, Gao L, Wang F, Xu J. "Steroid Receptor Coactivator-3 is a critical mediator in the development of castration-resistant prostate cancer." **Poster Presentation** the American Association for Cancer Research Annual Meeting, Chicago, 2012

Tien JC, Liu Z, Gao L, Wang F, Xu J. "Steroid Receptor Coactivator-3 is a critical mediator in the development of castration-resistant prostate cancer." **Poster Presentation** AACR Special Conference titled Advances in Prostate Cancer Research American, Orlando, 2012

Tien JC, Liu Z, Gao L, Wang F, Xu J. "Steroid Receptor Coactivator-3 is an Oncogene in the Setting of Pten Deletion." **Poster Presentation** at the Texas A&M Health Science Center IBT Student Research Symposium, Houston, TX. 2011

Oral Presentation

Tien JC, Liu Z, Gao L, Wang F, Xu J. "Steroid Receptor Coactivator-3 is an Oncogene in the Setting of Pten Deletion." **Oral Presentation** at Society of Chinese Bioscientists in America (SCBA) Symposium, Houston, TX. 2011

CONCLUSION:

Here, I simultaneously deleted Pten and SRC-3 using the ARR2PBi-Cre, which is expressed principally in prostatic luminal epithelial cells. From the results obtained, I conclude that inhibition of SRC-3 in the setting of Pten deletion yields smaller prostate tumor size with a more basal-like cell phenotype. This indicates SRC-3 plays an oncogenic role in tumors derived from luminal cells and that without SRC-3, mitotic ability of LEC-like tumor cells is impaired. During the course of studying the function of SRC-3 in castration-resistant prostate cancer, we found that although androgen deprivation shrunk the size of the tumor, the reduced level of testosterone accelerates prostate tumorigenesis, making castrated Pten-null tumors more aggressive and less differentiated with increased stromal reactivity. Amazingly, the androgen-deprived tumor phenotype is dependent on SRC-3, as SRC-3 deletion results in almost complete reversal of all castration-induced changesSRC-3 deletion also yields decrease in S6 kinase, a mediator of cellular translational output. Therefore, SRC-3 may mediate translational pathway that contributes to castration-resistant prostate cancer. In sum, SRC-3 is a critical mediator of advanced and castration-resistant prostate cancer. Design of SRC-3 inhibitor may be a novel therapeutic strategy for treating both tumor types.

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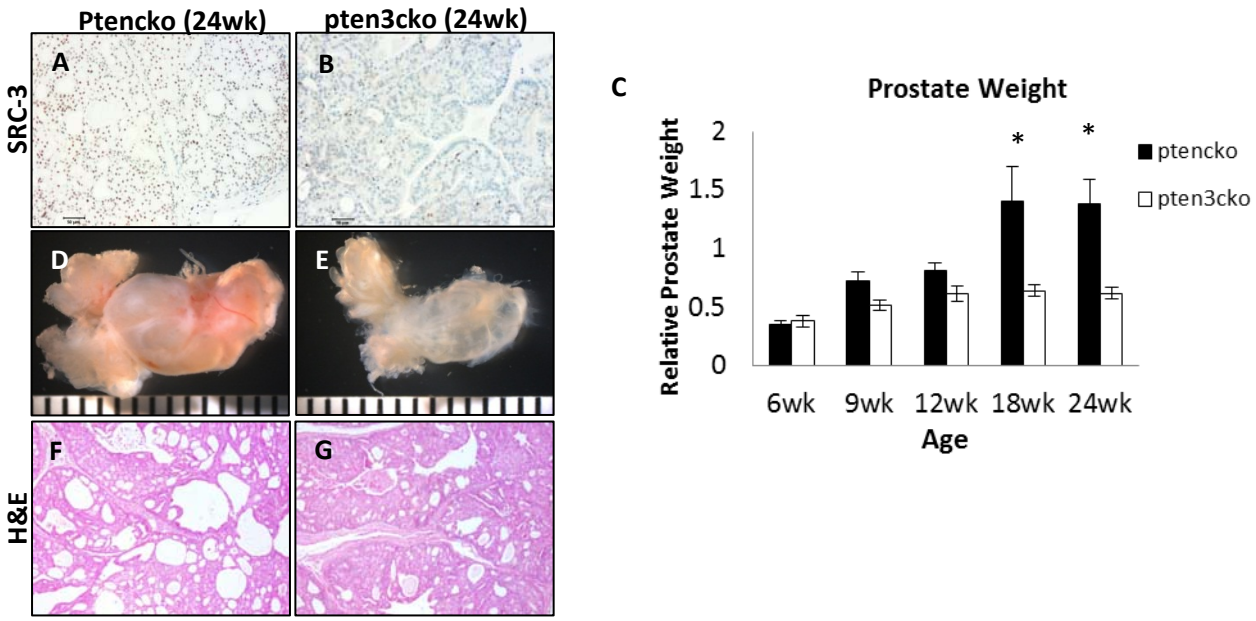
4. Yan, J., Yu, C. T., Ozen, M., Ittmann, M., Tsai, S. Y., and Tsai, M. J. (2006) *Cancer Res* **66**, 11039-11046

SUPPORTING DATA:

Figure 1



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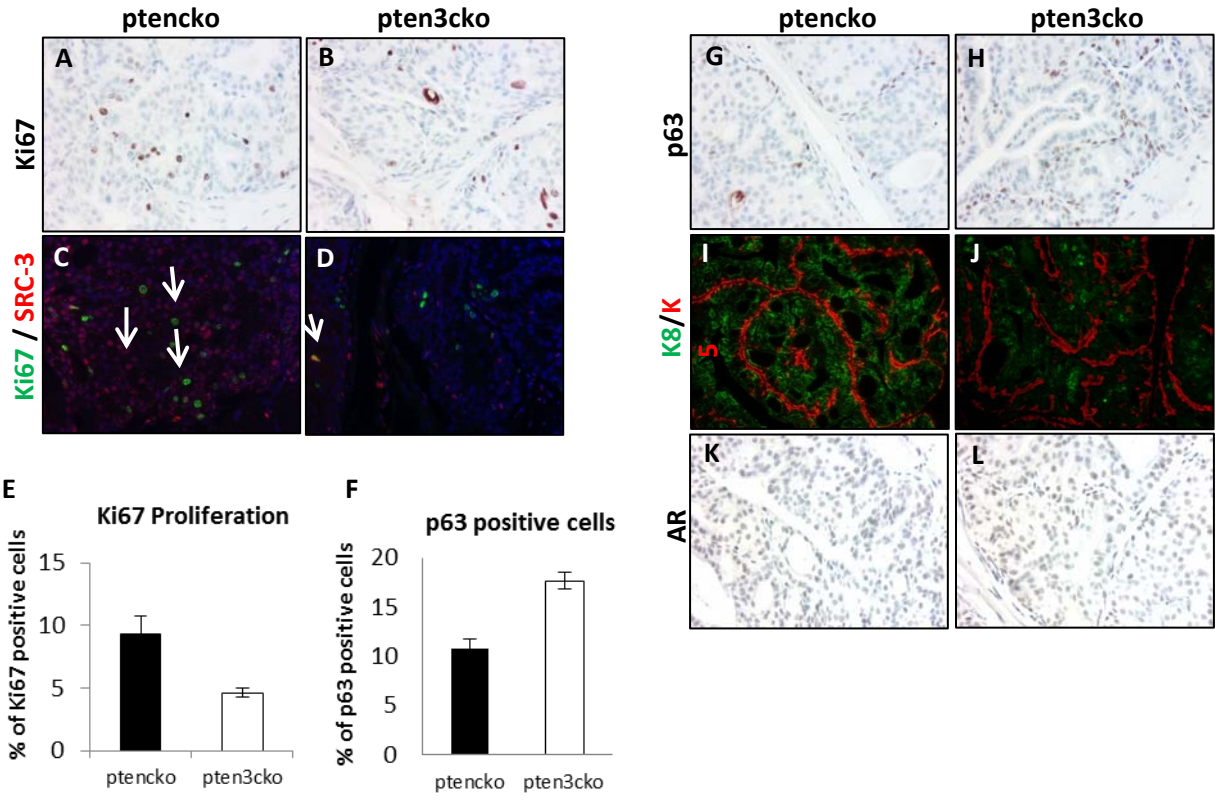
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B
Ki67
C
D
Ki67 / SRC-3
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5
0
ptencko
pten3cko
F
p63 positive cells
% of p63 positive cells
20
15
10
5
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I
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K8/K5
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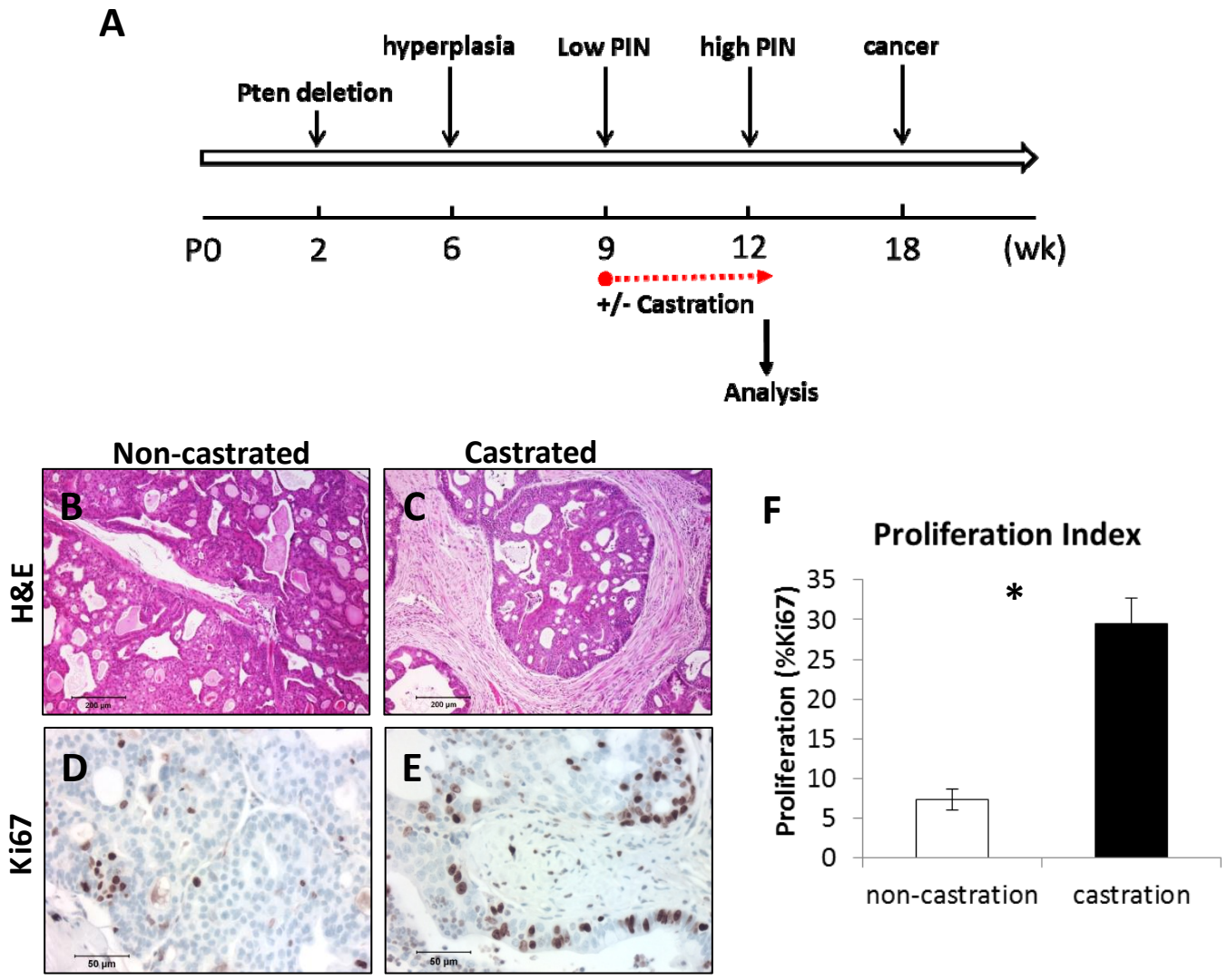
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high PIN
cancer
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9
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18
(wk)
+/- Castration
Analysis
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Castrated
H&E
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C
D
E
F
Proliferation Index
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20
15
10
5
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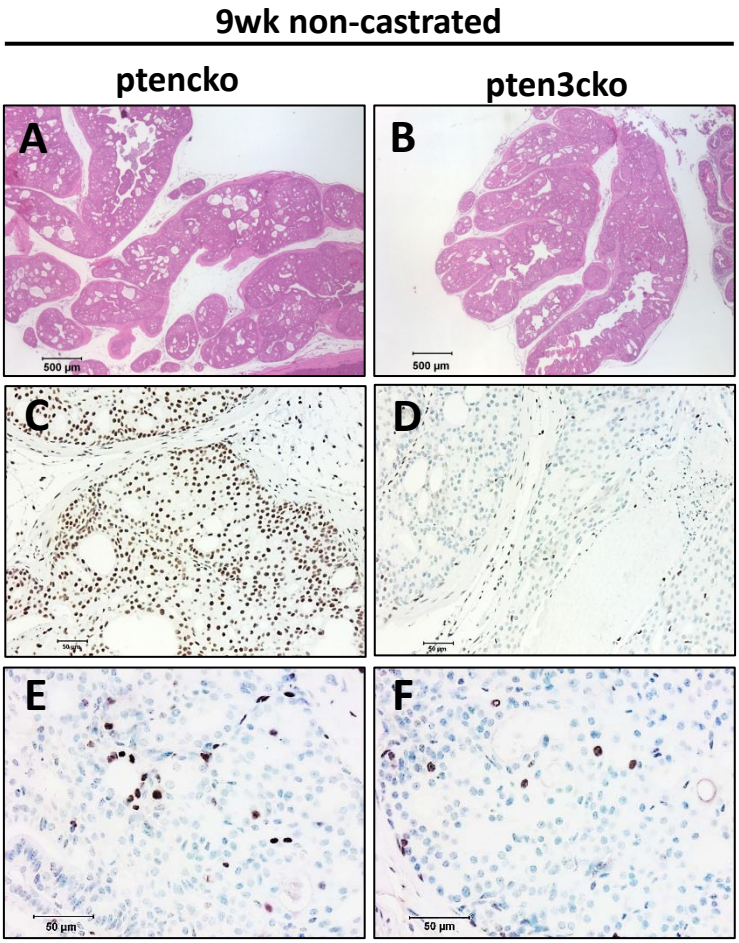
*
non-castration
castration

Figure 4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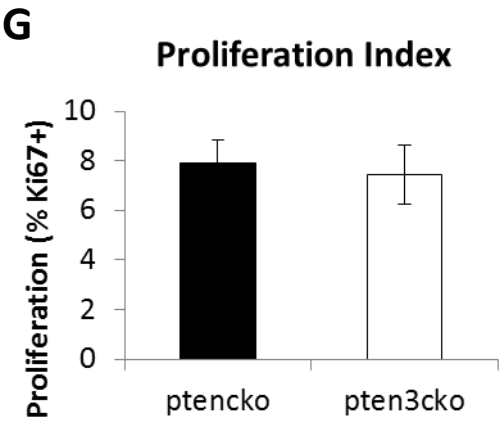
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Proliferation (% Ki67+)
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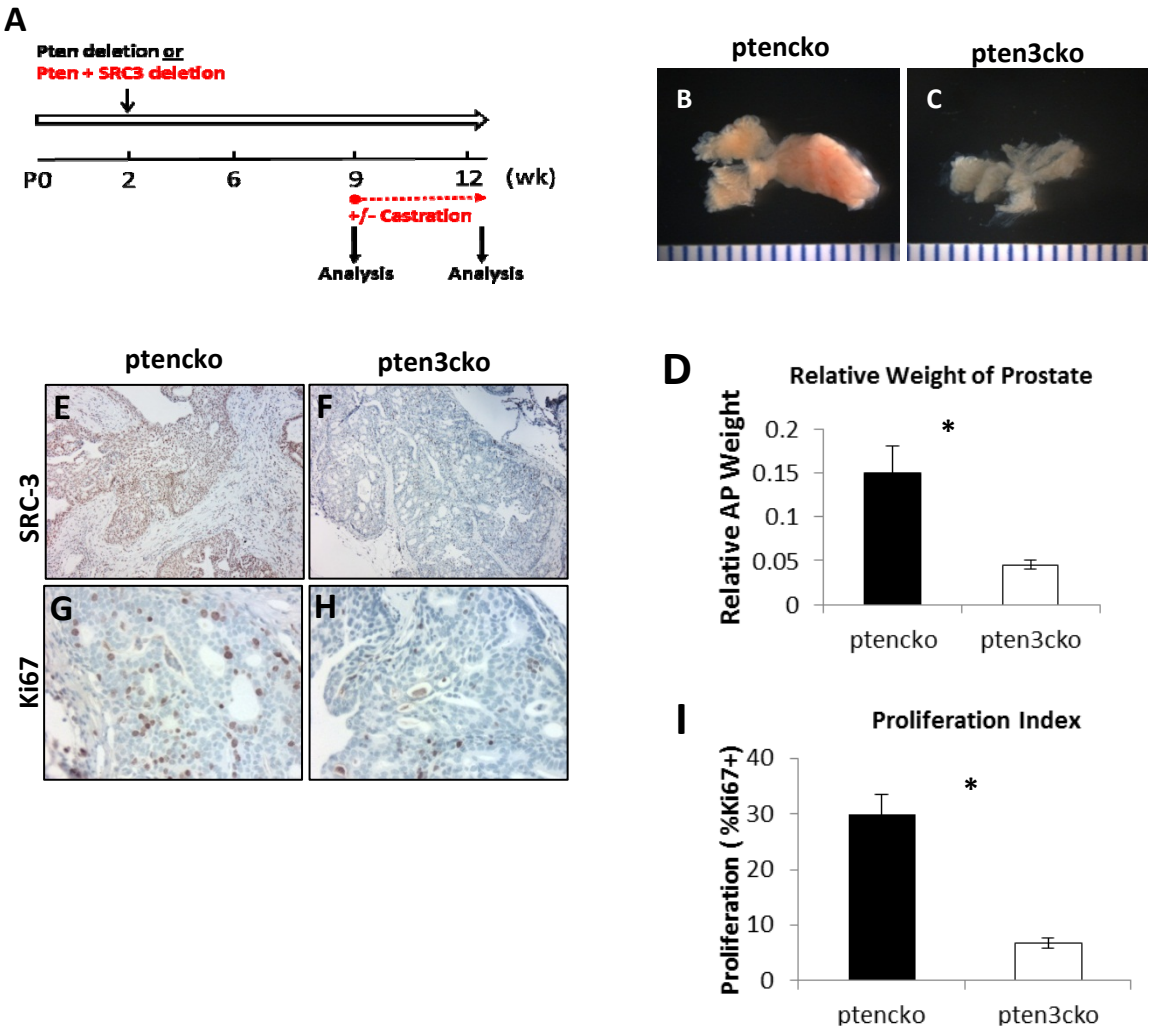
ptencko
pten3cko

Figure 5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Pten deletion or
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(wk)
+/- Castration
Analysis
Analysis
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pten3cko
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SRC-3
G
H
Ki67
D
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Relative AP Weight
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0.1
0.05
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Figure 6

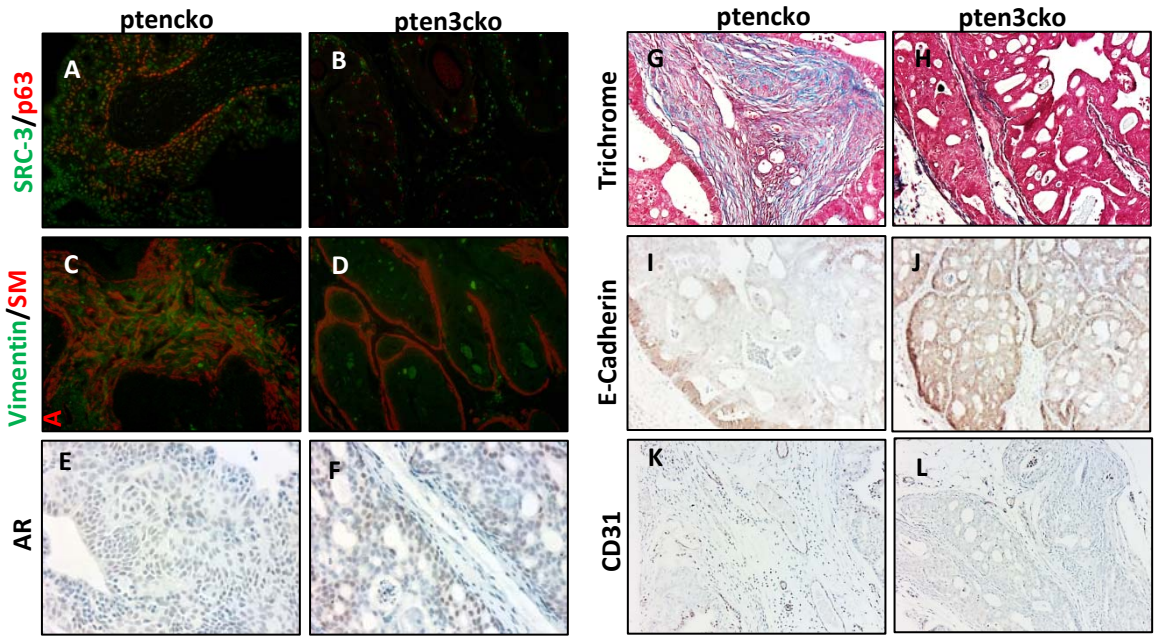
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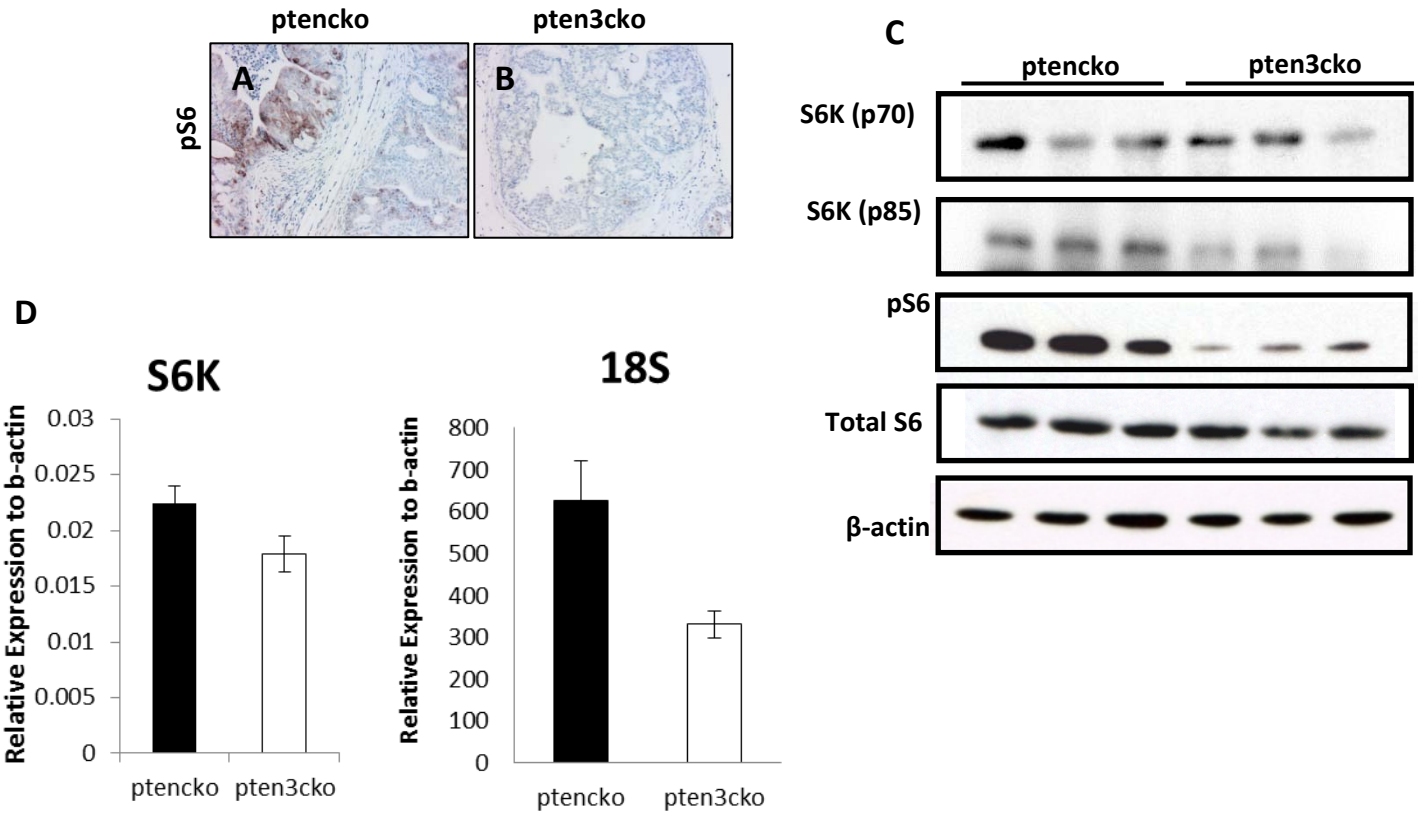
Figure 7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0.005
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ptencko
pten3cko
18S
Relative Expression to b-actin
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700
600
500
400
300
200
100
0
ptencko
pten3cko

AACR ANNUAL MEETING
March 31 - April 4, 2012 • Chicago, IL

Presentation Abstract

Abstract Number:	3296
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Presentation Time:	Tuesday, Apr 03, 2012, 8:00 AM -12:00 PM
Location:	McCormick Place West (Hall F), Poster Section 11
Poster Section:	11
Poster Board Number:	30
Author Block:	Jean C. Tien[1], Zhaoliang Liu[2], Li Gao[3], Fen Wang[1], Jianming Xu[3]. [1]Texas A&M Health Science Center, Houston, TX; [2]UT MD Anderson Cancer Center, Houston, TX; [3]Baylor College of Medicine, Houston, TX

Abstract Body:

Androgen ablation therapy is the standard treatment for advanced prostate cancer. While tumors initially respond to androgen deprivation, they almost always recur with an androgen-independent phenotype termed castration-resistant prostate cancer (CRPC). Castration-resistant tumors are incurable with current therapy regimens and account for most prostate cancer mortality. Better understanding of their biology is therefore critical for devising novel treatment options. Steroid Receptor Coactivator 3 (SRC-3) is a nuclear receptor coactivator that promotes growth of endocrine tissues. It enhances proliferation of prostate cancer cell lines and is highly expressed in advanced human prostate tumors. Despite this, the role of SRC-3 in CRPC is not well studied. PTEN is a tumor suppressor gene mutated in most human prostate cancers. Mice harboring PTEN deletion in prostatic epithelial cells develop cancer that arises from progenitors in the luminal epithelial and basal compartments. Tumors histologically mimic advanced human disease and are castration-resistant. **We hypothesized that, in prostate tumors caused by PTEN deletion, SRC-3 is a critical mediator for accelerating the development of CRPC.** To test this hypothesis, we generated mice in which floxed *PTEN* and *SRC-3* genes were concomitantly deleted in prostate epithelial cells via a Cre recombinase driven by the Probasin promoter. We first compared tumor mass, histology and biomarkers in these $PTEN^{f/f}$/$SRC\text{-}3^{f/f}$/ARR2PBiCre (pten3cko) mice vs. $PTEN^{f/f}$/ARR2PBiCre (ptencko) mice. We found that while tumor growth in pten3cko mice was slightly inhibited at 9 wks of age, tumor histology and cellular composition were not markedly different. We then castrated mice of both genotypes at 9-wks-old and harvested tumors at 12wks. Interestingly, castrated ptencko mice had significantly worsened tumor aggressiveness--as indicated by increased proliferation index, reduced differentiation (increased p63; decreased K5 and K8) and increased reactive stroma (double staining of SMA and vimentin)--versus their non-castrated counterparts. Amazingly, in addition to significantly reducing tumor size, SRC-3 ablation reversed all changes comprising the aggressive phenotype seen in the post-castration tumors. Further investigation showed SRC-3 deletion decreased mTOR signaling. Together, these data show that castration induces a worse tumor phenotype in the setting of PTEN deletion and that SRC-3 promotes this process by inducing epithelial cell proliferation and enhancing the growth-promoting effect of these cells on the stroma. Therefore, SRC-3 may serve as a potential target for controlling CRPC development.

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